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(54) Title: METHODS AND COMPOSITIONS FOR DIETARY SUPPLEMENTS

(57) Abstract: Compositions and methods are provided to reduce glucose concentration in an organism. Particularly contemplated compositions include a compound that binds to a thaumatin-like protein and that is isolated from a plant. Contemplated compositions further reduce blood lipid concentrations at the concentration effective to reduce the glucose concentration.

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METHODS AND COMPOSITIONS FOR DIETARY SUPPLEMENTS

Field of The Invention

The field of the invention is dietary supplements and related methods.

Background of The Invention

Elevated blood glucose and blood lipids are a relatively common underlying condition in numerous diseases and may be acquired in various ways. Among other causes, elevated blood glucose levels is frequently precipitated by an altered metabolism associated with a diabetic condition, and treatment of diabetic conditions often includes insulin therapy along with synthetic oral anti-diabetic agents, such as metformin, sulfonylurea, etc. Despite an improvement of some clinical parameters (*i.e.* reduction of blood glucose to at least some extent) in people with elevated blood lipid and blood glucose, various side effects, including insulin resistance, allergic reactions, etc. may arise from long-term treatment using insulin.

Alternative treatments of diabetes, and especially non-insulin dependent diabetes mellitus (NIDDM), are frequently based on yeast, or derivatives of yeast. Yeast can be grown in the presence of chromium salts, and yeast cells or extracts of cells grown in that manner are particularly rich in "glucose tolerance factor" (GTF), a compound known to enhance the biological effect of insulin. Although some yeast preparations help reducing elevated blood glucose concentrations, in many cases considerable amounts of yeast preparations must be ingested for a substantial period in order to improve a hyperglycemic condition. Moreover, long-term use of yeast preparations over extended periods tends to become problematic for some patients, especially where those patients have a history of yeast infections. Still further, many crude yeast preparations have a bitter taste that some patients may find objectionable.

To alleviate at least some of the problems associated with yeast preparations, concentrated, de-bittered and freeze dried yeast preparations have been developed. Such preparations are typically in tablet form, and may conveniently be ingested during a meal. However, the relatively high degree of processing of such cells/extracts may reduce the biological potency of the yeast preparation. Moreover, preservatives and additives (*e.g.*, for

pressing or otherwise forming of tablets) are typically needed to maintain at least some anti-hyperglycemic activity.

In still other methods of reducing blood glucose on a non-insulin basis, chromium picolinate may be administered. Chromium picolinate is reported to be moderately effective in reducing an elevated blood glucose level in human. However, chromium picolinate exhibits considerable toxicity and may therefore not be generally regarded as safe.

Although various methods of reducing an increased blood concentration of glucose are known in the art, all or almost all of them suffer from one or more disadvantages. Therefore, there is still a need to provide improved compositions and methods to reduce glucose concentration.

Summary of the Invention

The present invention is directed to compositions and methods of reducing glucose concentrations in an organism. More specifically, contemplated compositions comprise a compound that binds to a thaumatin-like protein and reduces a concentration of glucose in an organism when the compound is administered to the organism at a concentration effective to reduce the concentration of glucose.

In one aspect of the inventive subject matter, the compound is isolated from a plant, preferably a plant belonging to the family of *Poaceae*, and most preferably from *Hordeum vulgare*. Contemplated isolation procedures include malting, mashing, salt extraction, buffer extraction, ethanol extraction, anion exchange chromatography, and molecular sieving. Alternatively, contemplated compounds may be synthesized *de-novo* at least in part.

In another aspect of the inventive subject matter, contemplated compounds are hydrophobic, have a molecular weight of no more than 1000 Da, are soluble in a lipophilic solvent at a concentration of at least 10 mg per milliliter, and have a UV/VIS absorption maximum of about 260 nm. In especially preferred aspects, the composition further reduces the concentration of a blood lipid (*e.g.*, triglycerides, fatty acids, HDL-cholesterol, and LDLcholesterol), and in still further aspects of the inventive subject matter, the composition may further comprise a tocol, vitamins, or other dietary supplements which may or may not be active in regulation of blood glucose and/or blood lipids.

In a further aspect of the inventive subject matter, a method of reducing a glucose concentration in an organism comprises a step in which a composition is provided that includes a compound that binds to a thaumatin-like protein. In another step, contemplated compositions are administered to the organism in a dosage effective to decrease the concentration of glucose.

Various objects, features, aspects and advantages of the present invention will become more apparent from the following detailed description of preferred embodiments of the invention.

Brief Description of The Drawing

Figure 1 is a flow diagram showing an exemplary method of reducing blood concentration of glucose according to the inventive subject matter.

Figure 2 is a schematic showing an exemplary preparation of contemplated compounds and thaumatin-like proteins.

Figure 3A is a table depicting reduction of blood glucose concentrations in human volunteers using contemplated compositions according to the inventive subject matter.

Figure 3B is another table depicting reduction of blood glucose concentrations in human volunteers using contemplated compositions according to the inventive subject matter.

Figure 4A is a table depicting reduction of blood lipid concentrations in human volunteers using contemplated compositions according to the inventive subject matter.

Figure 4B is another table depicting reduction of blood lipid concentrations in human volunteers using contemplated compositions according to the inventive subject matter.

Figure 5 is a graph depicting fermentation rates of yeast incubated with contemplated compounds at anaerobic and aerobic conditions.

Detailed Description

As used herein the term "compound that binds to a thaumatin-like protein" refers to any compound or mixture of compounds that exhibit a binding preference to a thaumatin-like protein from barley of at least 10-fold, more preferably at least 100-fold over binding to other barley proteins, wherein binding of contemplated compounds to the thaumatin-like protein will preferably have a K_D of less than 10^{-3}M, more preferably of less than 10^{-4}M. The mode of binding need not be limited to a single interaction (*e.g.*, hydrophobic interaction), but may include multiple interactions (*e.g.*, electrostatic interactions and hydrogen bonding, etc.). It is especially contemplated that binding is reversible, however, irreversible binding is not excluded. Although thaumatin-like proteins from barley are generally preferred binding partners for compounds according to the inventive subject matter, thaumatin-like proteins from alternative sources, including microorganisms, plants, and animals are also contemplated. Thaumatin-like proteins are a well characterized class of polypeptides and are described, for example, in *Cvetkovic et al.*, J. Serb Chem. Soc. 62(9):777-786 (1997), *Cvetkovic et al.*, J. Serb. Chem. Soc. 62(1):51-56 (1997) and *Cvetkovic et al.*, J. Inst. Brew. 103:183-186 (1997), all of which are incorporated by reference herein.

As also used herein, the term "elevated glucose concentration" refers to a concentration that is above the clinical range considered normal (*i.e.*, above 110 mg/dl). Similarly, the term "elevated lipid concentration" refers to a concentration of blood lipids that is above the clinical range considered normal.

In **Figure 1**, a method 100 of reducing a glucose concentration in an organism has a step 110 in which a composition is provided that includes a compound that binds to a thaumatin-like protein. In a subsequent step 120, the composition is administered to the mammal in a dosage effective to decrease the blood concentration of glucose.

In an especially preferred aspect of the inventive subject matter, the composition is prepared from *Hordeum vulgare* (as outlined in examples, *infra*), and orally administered in 3 daily doses of 500mg, respectively, to a human diagnosed with non-insulin dependent diabetes mellitus (NIDDM). Thus, especially preferred compositions include a compound

that binds to thaumatin-like proteins and that reduces a concentration of glucose in an organism when the compound is administered to the organism at a concentration effective to reduce the concentration of glucose.

In alternative aspects of the inventive subject matter, it is contemplated that appropriate compositions and compounds need not be limited to a preparation from *Hordeum vulgare*, but may also include preparations from various plants other than *Hordeum vulgare*, and particularly contemplated alternative plants include *Hordeum spec.*, and members of the *poaceae* family. While the preparation of contemplated compositions and/or compounds is preferably from plant extracts, it should further be appreciated that contemplated compositions and/or compounds may also be isolated from microorganisms (*i.e.*, bacteria, fungi, yeasts, unicellular eucaryotic organisms) or animals, so long as contemplated compounds bind to a thaumatin-like protein and reduce a glucose concentration in an organism.

In still further alternative aspects, it should be appreciated that contemplated compounds may be isolated, purified to homogeneity, and the structure be elucidated. Consequently, it should be appreciated that contemplated compounds and/or compositions may be entirely (*de novo*) or partially synthesized/modified *in vitro*. For example, where contemplated compounds are partially synthesized, a precursor of contemplated compounds may be isolated from a plant or microorganism, and then be subjected to one or more steps to arrive at contemplated compounds. Alternatively, contemplated compounds may be modified in one or more synthetic steps to impart a particularly desirable physico-chemical property. For example, contemplated compounds may be esterified with a polar compound (*e.g.* polyethylene glycol) to increase water solubility. In another example, contemplated compounds may be coupled to a resin or other material to control the rate of release to the organism.

Preferred contemplated compounds have a relatively low molecular weight, typically no more than 1000Da, however, it should be recognized that the molecular weight may vary considerably and will predominantly depend on the source from which the compound is isolated, synthetic modifications, dimerizations and multimerizations. Likewise, it is contemplated that suitable compounds need not be limited to compounds having a UV

absorption maximum at about 260nm (which is characteristic for contemplated compounds isolated using the procedure outlined below), and various spectral characteristics other than a UV_{260} peak are also suitable. Similarly, while contemplated compounds isolated from *Hordeum vulgare* are soluble in a lipophilic solvent at a concentration of at least 10mg per milliliter, higher or lower solubilities are also contemplated and will typically depend on the source from which contemplated compounds are isolated, and/or on further chemical modifications of contemplated compounds. The term "lipophilic solvent" as used herein includes all solvents that have a miscibility with H_2O of less than 10vol%.

While it is generally preferred that contemplated compounds are chemically substantially pure (*i.e.*, concentration of contemplated compounds greater than 90wt%, preferably greater than 95wt%, most preferably greater than 99wt%), it should also be appreciated that contemplated compounds may be coupled to one or more than one molecule, and particularly contemplated molecules include thaumatin-like proteins. Thus, contemplated compositions include complexes between contemplated compounds and thaumatin-like proteins, and especially include complexes between contemplated compounds and thaumatin-like proteins as they are isolated from the appropriate sources (*infra*).

With respect to the glucose concentration, it is generally contemplated that the glucose concentration is a blood glucose concentration. However, further contemplated glucose concentrations also include concentrations of glucose covalently or non-covalently bound to molecules found within the organism, and especially contemplated alternative glucose concentrations include concentrations of glycosylated proteins (*e.g.*, glycosylated hemoglobin or collagen).

While it is generally contemplated that suitable thaumatin-like proteins are isolated from *Hordeum vulgare*, alternative thaumatin-like proteins are also contemplated and include thaumatin-like proteins isolated from microorganisms, plants, and animals, which may or may not be expressed in a recombinant system. There are various protocols for isolation of thaumatin-like proteins known in the art (see *e.g.*, Barre et al, Purification and structural analysis of an abundant thaumatin-like protein from ripe banana fruit. Planta. 2000 Nov;211(6):791-9; Oh, et al., Isolation of a cDNA encoding a 31-kDa, pathogenesis-related

5/thaumatin-like (PR5/TL) protein abundantly expressed in apple fruit. Biosci Biotechnol Biochem. 2000 Feb;64(2):355-62; Tattersall, et al. Identification and characterization of a fruit-specific, thaumatin-like protein that accumulates at very high levels in conjunction with the onset of sugar accumulation and berry softening in grapes. Plant Physiol. 1997 Jul;114(3):759-69), and all the known protocols are considered suitable for use in conjunction with the teachings presented herein.

It should be especially appreciated that contemplated compositions not only reduce elevated blood glucose concentration in human suffering from NIDDM, but may also reduce blood glucose concentrations in individuals having elevated blood glucose concentrations for reasons other than NIDDM, including obesity, dietary effects, etc. It is especially contemplated that individuals with or without NIDDM will have a blood glucose concentration of at least 90mg/dl, more preferably of at least 120 mg/dl, and most preferably of at least 200 mg/dl.

Furthermore, contemplated compositions have also been shown to advantageously reduce elevated blood lipid concentrations (*infra*), wherein blood lipids particularly include triglycerides, fatty acids, HDL-cholesterol, and LDL-cholesterol, and it is further contemplated that the reduction of blood lipids may be concomitantly with the reduction of blood glucose levels, or independent of the reduction of the blood glucose level.

In further aspects of the inventive subject matter, it should be appreciated that contemplated compositions may further comprise active or inactive ingredients, including compositions known to decrease a blood lipid concentration, and/or compositions known to decrease blood sugar concentrations. For example, alternative compositions may include at least one of a tocol, vitamins, and/or mineral preparations, GTF, metformin, sulfonylurea, and the like. Inactive ingredients include fillers, coloring agents, stabilizers, and the like.

Thus, an exemplary method of treating a person (*e.g.*, diagnosed with NIDDM) having an increased blood concentration of glucose of approximately 150 mg/dl, and an increased blood concentration of total cholesterol of above 280 mg/dl, or more has one step in which contemplated compositions are provided. In a further step, the composition is administered to the person in a dosage effective to decrease the concentration of glucose.

With respect to the blood glucose level it is contemplated that a treatment according to the inventive subject matter need not be limited to blood glucose levels of approximately 150 mg/dl, but may also be indicated at many blood concentrations of glucose above 70-110 mg/dl. Although not wishing to be bound to a particular theory or mechanism, it is contemplated that the reduction in the blood glucose level may be due to an enhanced glucose uptake into the cell. However, it should be noted that compositions according to the inventive subject matter are non-GTF compositions. The duration for contemplated treatments may vary significantly, and suitable durations may be within the range of a single dose, but also for a predetermined period, including one week, several weeks, several months, and even several years. Consequently, it be appreciated that compositions according to the inventive subject matter may also be prophylactically administered to a human to prevent hyperglycemia, or some form of dyslipidemia.

In further alternative aspects of the inventive subject matter, the composition may also be administered to an organism other than a human, and particularly preferred alternative organisms include livestock (*e.g.*, cattle, pigs, horses, etc.) and pets (*e.g,* dogs, cats, rodents, birds, etc.). With respect to contemplated compositions, the same considerations as described above apply.

It is especially contemplated that treatment according to the inventive subject matter may also result in significant weight loss, particularly in persons with obesity, NIDDM, or other condition associated with increased body weight. It is generally contemplated that the treatment according to the inventive subject matter is not limited to reduction of blood glucose alone, but may concomitantly (or by itself) include reduction of a particular lipid or lipid group. For example, slightly elevated total cholesterol (*e.g.*, 220 mg/dl) may be an indication for treatment with the contemplated compounds. Alternatively, it is contemplated that an imbalance between HDL and LDL (*i.e.* LDL>>HDL) may be normalized employing a treatment according to the inventive subject matter. Similarly, while the total cholesterol in the patient need not be elevated, treatment with the contemplated method may still be indicated due to an elevated triglyceride level.

With respect to the dosage, form, and route of administration it is contemplated that there are many alternative oral preparations besides 3 oral daily doses of 500mg. For

example, where relatively high dosages are required, dosages may increase from 500mg – 5g per day, and more. High dosages may also be required where the potency of an extract is relatively low. Likewise, in cases where low dosages (*e.g.*, maintenance therapy) are required, or the extract has a comparably high potency, daily dosages between 500mg and 25mg, or less, are appropriate. Therefore, it is generally contemplated that among other parameters the patient's particular condition and the potency of the preparation will at least partially determine the frequency of application. For example, where high dosages are to be administered to the patient, more than 3 daily dosages are contemplated, including 4-6 and more. Where low dosages, especially dosages lower than 500mg/day are contemplated, single, bidaily, or less frequent administrations are appropriate.

Of course it should also be recognized that the form of administration may vary considerably. For example, oral administration need not be limited to a tablet, and alternative oral administrations may include powders, gel-caps, syrups, gels, etc. Where oral administration is not desirable, it is further contemplated that alternative routes are also appropriate, including injections, transdermal, pulmonary or intranasal delivery.

Examples

The following examples provide various experimental procedures to make and use contemplated compounds according to the inventive subject matter. Examples 1 and 2 describe basic and improved procedures of producing compositions according to the inventive subject matter, respectively. The biological activity of the compounds isolated according to procedures in Examples 1 and 2 is described in Example 3 and 4, and Example 5 provides experimental support for specific binding of contemplated compounds to thaumatin-like proteins.

Example 1

Barley grains were malted according to procedures well known in the art of beer brewing (see *e.g.*, Principles of Brewing Science, Second Edition, by George J. Fix; Brewers Publications; ISBN: 0937381748, or The Brewers' Handbook by Ted Goldhammer; KVP Publishers; ISBN: 0967521203). In order to extract soluble substances from the malt and to

convert additional insoluble solids into soluble material through controlled enzymatic conversion, a step of mashing was subsequently applied to the ground malt (suspended in water) according to a typical brewer's schedule. The temperature cycles were as follows: Incubation at 40°C for 60min, incubation at 50°C for 60 min, incubation at 60°C for 60 min, incubation at 72°C for 60 min, and incubation at 75°-80°C for 60 min. Soluble portions of samples were separated from husks and other insoluble material and freeze-dried.

The freeze-dried barley extract obtained after mashing at 40°C served as base for fractionation into its components. A first fractionation was achieved by preparative liquid chromatography using a DEAE-Sephacel column (2.6 x 20 cm) equilibrated with 50mM phosphate buffer, pH 7.8. 150 mg of the freeze-dried sample was dissolved in 10 ml of buffer and placed on the column. A linear NaCl-gradient (0 - 0.5 M) was run at a flow rate of 10 ml/h. Fractions (2 ml each) were collected, and elution was monitored at 280 nm. The DEAE chromatography resulted in four distinct protein peak fractions: I – basic, II – neutral, III- and IV – acidic. Respective peak fractions were collected, desalted and concentrated by membrane ultra-filtration using a membrane cut–off pore size of 1000 Dalton, and concentrated corresponding fractions were checked for their capacity to influence yeast fermentation rate. The basic fraction I produced significant inhibitory effect (*i.e.*, a reduction of the yeast fermentation rate), while the remaining three concentrated fractions were almost inert. As it could later be identified (data not shown), the main proteinaceous component in fraction I represent thaumatin-like proteins. It has been noticed during the membrane ultra-filtration of the pooled protein fractions I – IV (*i.e.*, fractions obtained by ion exchange chromatography), that the filtrate of some fractions contains LMW (low molecular weight) substances with a UV absorbance maximum of approximately 260 nm. These observations prompted us to employ molecular sieving chromatography to separate these LMW substances from proteins in these fractions.

For that purpose, the four separated fractions by DEAE-Sephacel column I-IV were pooled and freeze-dried. Molecular sieving chromatography was performed on Sephadex G-75-50 column (2.8 x 80 cm) with 50 mM phosphate buffer, pH 7.8, containing 0.5 M NaCl (flow rate – 12 ml/h, fractions 2 ml, elution recorded at 260 nm). LMW compounds with an absorbance near 260 eluted at relatively high elution volume. Where the separated fractions were individually subjected to molecular sieving on a Sephadex G-75-50 column, LMW

compounds eluted near to the end of the separation, typically between 60th – 80th fractions. These fractions were designated GMM-1, GMM- 2 and GMM-4, and consist of LMW components.

All of GMM-1, GMM- 2 and GMM-4 enhanced yeast fermentation, bound strongly and reversibly to thaumatin-like protein (bind to thaumatin-like proteins at low salt condition and release from thaumatin-like proteins at high salt condition), and reduced elevated blood glucose concentration and elevated blood lipid concentration in human diagnosed with NIDDM.

Example 2

20 g of malted barley flour was suspended in 80 ml of water and stirred over night at ambient temperature. The suspension was supplemented with 120 ml of 0.8 M NaCl solution and salt extraction was continued for 24 hours with stirring. An aqueous extract was separated from the suspension by vacuum filtration over a cellulose filter pad. Alternatively, citrate or other buffers are also contemplated suitable for preparation of an aqueous extract.

The filtered extract was freeze-dried or vacuum-evaporated. So obtained dry malt extract (yield approx. 12–14 g) contained 5.6 g of NaCl originating from the extracting solvent and a complex mixture of water-soluble barley components. The filtered freeze-dried extract was purified by extraction with two 50 ml portions of warm ethanol under vigorous mixing for two hours. The ethanolic extracts were filtered, combined, and evaporated to an oily residue in vacuum. The oily residue was re-dissolved in 15 ml of water and freeze-dried, resulting in a hard glassy yellowish product in a total amount of approx. 3 g.

The glassy yellowish product enhanced yeast fermentation, bound strongly and reversibly to thaumatin-like protein (bind to thaumatin-like proteins at low salt condition and release from thaumatin-like proteins at high salt condition), and reduced elevated blood glucose concentration and elevated blood lipid concentration in human diagnosed with NIDDM.

Thus, it should be recognized that contemplated compositions comprise a plant seed extract (preferably from *Hordeum vulgare*), wherein the plant seed is malted (preferably at a

temperature between about 30°C and 65°C) and the extract is prepared from the malted plant seed using a protocol that includes an aqueous extraction step (*e.g.*, using an aqueous buffer such as a citrate buffer), and that the extract reduces a glucose concentration in an organism when the extract is administered to the organism at a concentration effective to reduce the concentration of glucose.

Example 3

The biological activity of LMW fractions from Example 1 (GMM-1, GMM- 2 and GMM-4) and the glassy yellowish product from Example 2 was monitored by quantification of brewers' yeast fermentation rate under anaerobic conditions using a modified Warburg method (Mirsky, N. et al., J. Inorg. Biochem. 13(1):11-21 (1980), which is incorporated by reference herein.

Two grams of wet brewers yeast cells (about 20% dry weight) were suspended in fermentation medium (25 ml of 60 mM phosphate buffer, pH 5.7 and 10 ml of 5% (w/v) glucose solution), and aliquots of the products from example 1 or 2 were added to the fermentation medium for testing. Incubations were carried out in 50ml fermentation flasks at 25°C for 60 minutes. The fermentation rates were measured from the volume of generated CO_2. All of the tested LMW fractions or the product from Example 2 showed significant biological activity or bioactivity in that they increased the yeast fermentation rate in the range of about 20 - 40%. As used herein, a bioactive compound is one that increases or decreases fermentation. In a further experiment, the activity of GMM-2 was checked at aerobic conditions. Despite general restriction of yeast fermentation caused by combined effects of NaCl from buffer and air oxygen (Pasteur effect), the relative amount of generated CO_2 was doubled in comparison to the included control. The comparative results for GMM-2 fraction at anaerobic and aerobic conditions are shown below in **Figure 5**. The results conclusively prove modulating activity of the isolated LMW substances on yeast metabolism.

Example 4

The product obtained in Example 2 was examined for use in humans diagnosed with NIDDM. 25 men were recruited from an outpatient clinic (Endocrinology Department).

Mean age within the group was 51 yr, ranging from 36 to 74. Medical records were screened to exclude diabetics taking insulin or oral hypoglycemic agents. All of the subjects agreed to maintain their usual eating habits and health-related behaviors throughout the study. The experimental treatments were run over a period of six month. The participants were instructed to take the preparation in 3 oral daily doses of 1,000 mg each in a tablet form.

All subjects were tested for plasma glucose, glucosylated hemoglobin HbAc1, triglycerides and cholesterol before supplementation and throughout the study at biweekly or monthly intervals depending on type of tests. The subjects were subdivided into groups according to patterns given below:

Plasma glucose: According to the plasma glucose levels the subjects were subdivided in three groups for differentiation of the effects: I - up to 8 mMol/L; II - 8 - 10.5 mMol/L and III - above 10.5 mMol/L of plasma glucose concentration. Glycosylated hemoglobin (HbAc1): According to the HbAc1 levels the subjects were divided in two groups: I - below 10% and II - above 10% of the modified hemoglobin. The test results related to glycemia, before and after treatment, are shown in **Figure 3A**.

A further set of clinical studies was performed with 10 human volunteers following a similar protocol as outlined above. In this second experiment, blood glucose was measured fasting and postprandial over a period of 90 days, and the results are shown in **Figure 3B**. As can be clearly seen, administration of contemplated compounds results in a decrease of fasting and/or postprandial blood glucose of at least 5%, more typically of at least 10%, and most typically of at least 20%. Similarly, the levels of glycosylated hemoglobin was reduced after administration of contemplated compounds at least 5%, more typically at least 20%, and most typically at least 50%.

The lipid status of the subjects diagnosed with NIDDM was determined before and after treatment by testing plasma level of triglycerides, and cholesterol (as total, LDL and HDL form). The test results shown in **Figures 4A** and **4B** include subjects with disturbed lipid metabolism due to diabetic disease.

The lipid status of the subjects as shown in Figure 4A includes plasma levels of triglycerides, the ratio of triglycerides over total cholesterol, and the ratio of LDL/HDL. The

latter two ratios are known as atherosclerotic risk factors. As can be seen from Figure 4A, administration of contemplated compounds resulted in a reduction of triglycerides of up to 50%, and a significant reduction of about 1-20% of the ratio of triglycerides to HDL cholesterol, with an even more dramatic reduction of the ratio between LDL to HDL cholesterol (about 40%). The lipid status as shown in Figure 4B includes further results of ten test patients after administration of contemplated compounds and/or compositions over a period of 90 days.

Example 5

Thaumatin-like proteins were prepared following the procedure as generally outlined in Example 1 and Figure 2. So isolated thaumatin-like proteins were subjected to repeated molecular sieving in a membrane concentrator using a membrane with a molecular weight cut off of about 1000Dalton. After a first round of filtration of the protein preparation, 99ml of buffer (50 mM phosphate buffer, pH 7.8, 0,5 M NaCl) were added to about 1ml of retentate (*i.e.* the thaumatin-like protein fraction), and three subsequent rounds of filtration were performed with the same buffer to remove remaining GMM-compounds (*i.e.*, herein presented compounds that reduce elevated glucose) from the thaumatin-like protein preparation. UV absorbance of the filtrate was monitored at 260nm and the biological activity of sample volumes from the filtrate was tested according to protocols outlined in Example 3. Such prepared thaumatin-like proteins were desalted by membrane filtration employing NaCl-free buffer (50 mM phosphate buffer, pH 7.8), and further used in the following procedure:

To 1ml of a desalted thaumatin-like protein solution (10 mg/ml), 1.0 ml of a GMM-1 solution (1mg/ml) was added, and the mixture was incubated at room temperature for 2hrs. After 2 hrs, 98 ml of 50 mM phosphate buffer, pH 7.8 were added to the mixture and unbound GMM-1 was removed by 3 subsequent rounds of ultrafiltration (each round 1:100 by volume) with buffer.

The thaumatin-like protein with the bound GMM-1 was labeled Sample 1. Sample 1 was then subjected to a molecular sieving chromatography using a Sephadex G-75 column with 50 mM phosphate buffer, pH 7.8, 0.5 M NaCl as solvent, in which a low molecular weight fraction eluted with an absorbance of 260nm separate from a higher molecular

weight fraction of the thaumatin-like protein with absorbance of 280nm. The low molecular weight fraction was concentrated, desalted, and brought to a volume of 1.0ml and labeled Sample 2. Samples 1 and 2 were then tested for biological activity employing a procedure as outlined in Example 3. While Sample 1 did not increase the rate of fermentation, Sample 2 significantly increased the rate of fermentation in both aerobic and anaerobic experimental conditions, thereby clearly demonstrating the reversible binding of GMM-1 to a thaumatin-like protein. The same procedure was repeated with GMM-2 and GMM-4. The obtained results were similar to the presented GMM-1 experiment.

Thus, specific embodiments and applications of compositions and methods to reduce glucose concentrations in an organism have been disclosed. It should be apparent, however, to those skilled in the art that many more modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the appended contemplated claims. Moreover, in interpreting both the specification and the contemplated claims, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms "comprises", and "comprising", should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

CLAIMS

We claim:

1. A composition comprising:

a compound that binds to a thaumatin-like protein and reduces a concentration of glucose in an organism when the compound is administered to the organism at a concentration effective to reduce the concentration of glucose.

2. The composition of claim 1 wherein the compound is isolated from a plant.

3. The composition of claim 2 wherein the plant is a *poaceae* plant.

4. The composition of claim 3 wherein the plant is *Hordeum vulgare*.

5. The composition of claim 2 wherein the compound is isolated using at least one procedure selected from the group consisting of malting, mashing, salt extraction, buffer extraction, ethanol extraction, anion exchange chromatography, and molecular sieving.

6. The composition of claim 1 wherein the compound has a molecular weight of no more than 1000 Da.

7. The composition of claim 6 wherein the compound has an UV absorption maximum of about 260 nm.

8. The composition of claim 7 wherein the compound is soluble in a lipophilic solvent at a concentration of at least 10 mg per milliliter.

9. The composition of claim 1 wherein the compound is synthesized *de novo*.

10. The composition of claim 1 wherein the thaumatin-like protein is isolated from barley.

11. The composition of claim 1 further comprising a tocol.

12. The composition of claim 1 wherein the compound further reduces a blood concentration of a lipid at a dosage effective to decrease the concentration of glucose.

13. The composition of claim 12 wherein the lipid is selected from the group consisting of a triglyceride, a fatty acid, an HDL-cholesterol, and an LDL-cholesterol.

14. The composition of claim 1 wherein the organism is a mammal.

15. The composition of claim 14 wherein the mammal is diagnosed with non-insulin dependent diabetes mellitus.

16. The composition of claim 14 wherein the concentration of glucose comprises a blood concentration of glucose, and wherein the blood concentration of glucose is greater than 120 mg/dl.

17. The composition of claim 16 wherein the blood concentration of glucose is greater than 200 mg/dl.

18. A composition comprising:

a plant seed extract, wherein the plant seed is malted and the extract is prepared from the malted plant seed using a protocol that includes an aqueous extraction step; and

wherein the extract reduces a glucose concentration in an organism when the extract is administered to the organism at a concentration effective to reduce the concentration of glucose.

19. The composition of claim 18 wherein the plant seed is a *Hordeum vulgare* seed.

20. The composition of claim 18 wherein the malting is performed at a temperature between 30°C and 65°C.

21. The composition of claim 18 wherein the extraction step includes extraction with an aqueous buffer.

22. The composition of clam 18 wherein the glucose concentration comprises a blood glucose concentration and wherein the organism is a human.

23. A method of reducing a concentration of glucose in an organism, comprising:

providing a composition that includes a compound that binds to a thaumatin-like protein; and

administering the composition to the organism in a dosage effective to decrease the concentration of glucose.

24. The method of claim 23 wherein the organism is a human.

25. The method of claim 24 wherein the human is diagnosed with non-insulin dependent diabetes mellitus.

26. The method of claim 23 wherein the concentration of glucose comprises a concentration of glucose in blood.

27. The method of claim 26 wherein the concentration of glucose in blood is greater than 120 mg/dl.

28. The method of claim 26 wherein the concentration of glucose in blood is greater than 200 mg/dl.

29. The method of claim 23 wherein the compound is isolated from a plant.

30. The method of claim 29 wherein the plant is *hordeum vulgare.*

31. The method of claim 23 wherein the compound has a molecular weight of no more than 1000 Da, is soluble in a lipophilic solvent at a concentration of at least 10 mg per milliliter, and has an UV absorption maximum of about 260nm.

32. The method of claim 31 wherein the compound is isolated using at least one procedure selected from the group consisting of malting, mashing, salt extraction, a buffer extraction, ethanol extraction, anion exchange chromatography, and molecular sieving.

33. The method of claim 23 wherein the compound is synthesized *de novo*.

34. The method of claim 23 wherein the step of administering comprises oral administration.

35. The method of claim 23 wherein the composition further reduces a blood concentration of a lipid at the dosage effective to decrease the concentration of glucose.

36. The method of claim 23 wherein the lipid is selected from the group consisting of a triglyceride, a fatty acid, a HDL-cholesterol, and a LDL-cholesterol.

37. The method of claim 23 wherein the composition further comprises a tocol.

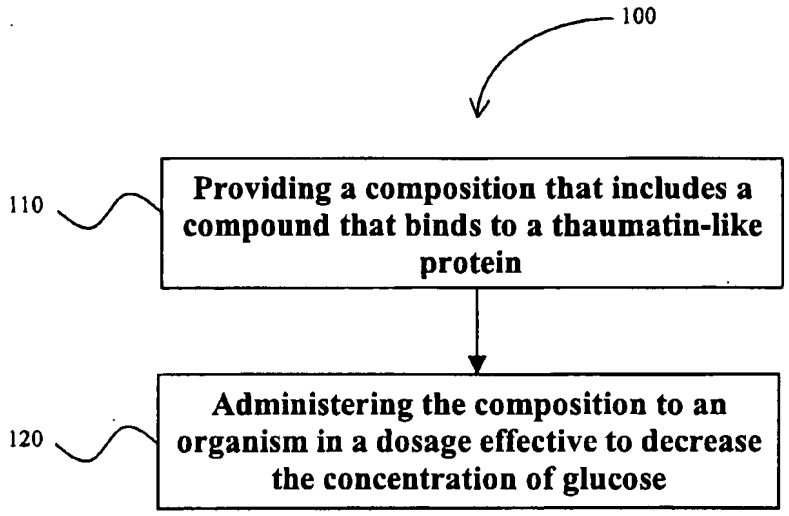
100
110
Providing a composition that includes a compound that binds to a thaumatin-like protein
120
Administering the composition to an organism in a dosage effective to decrease the concentration of glucose

Figure 1

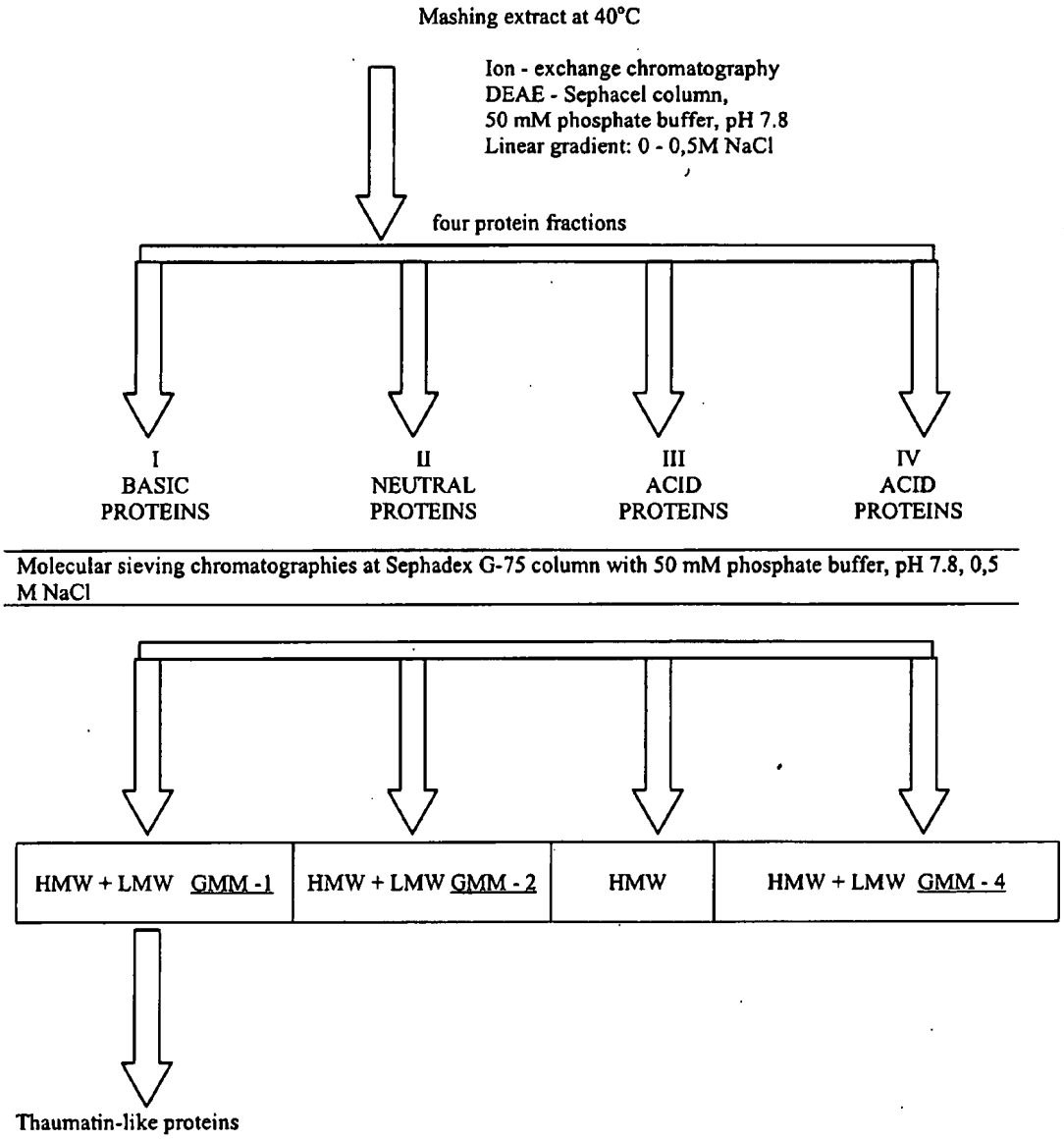

Figure 2

Group	No. of Patients	Before treatment		After treatment		Decrease (%)
		range	mean	range	mean	
Plasma Glucose (mMol/L)						
I	6	6.8-8.5	7.6	6.8-7.7	7.2	5.6
II	8	8.1-10.2	9.0	7.0-9.0	7.3	23.3
III	7	11.6-14.7	12.5	6.0-10.2	8.1	54.3
Glucosylated Hemoglobin-HbA1c(%)						
I	7	8.0-10.0	9.1	7.6-9.0	8.0	13.8
II	8	10.1-19.6	13.0	6.5-10.0	8.1	60.5

Figure 3A

Patient														
	0 days		15 days		30 days		45 days		60 days		75 days		90 days	
	F	PP	F	PP	F	PP	F	PP	F	PP	F	PP	F	PP
1	10.9	11.5	8.6	9.9	9.8	9.9	7.5	13.4	9.1	9.5	8.9	10.2	8.8	12.7
2	7.2	9.9	5.8	6.8	6.3	8.3	5.4	6.0	5.9	7.8	5.7	6.2	5.4	5.8
3	11.0	18.0	10.6	13.3	9.2	11.9	10.9	13.5	10.3	12.6	9.1	11.1	10.0	10.7
4	5.7	8.2	5.9	9.3	6.5	7.5	6.4	6.6	5.8	6.4	5.6	5.9	5.4	6.2
5	5.9	7.3	3.9	5.1	5.3	6.1	5.3	7.9	5.6	5.9	5.6	5.9	5.0	5.5
6	7.5	10.7	5.3	9.8	6.4	6.8	5.0	10.0	5.1	9.0	4.7	6.1	4.2	6.2
7	6.5	8.8	6.6	8.8	6.5	7.8	6.8	6.9	6.6	8.5	6.3	6.5	5.6	6.3
8	10.8	17.0	10.9	19.4	12.2	18.1	10.0	15.6	11.8	18.1	13.0	22.4	10.0	16.5
9	14.4	16.6	11.8	16.7	14.4	18.6	11.0	15.2	12.6	18.2	10.5	16.4	10.0	14.3
10	9.9	13.2	8.1	11.2	8.2	12.0	7.9	10.2	7.5	9.8	7.3	9.5	7.0	8.2

Figure 3B

No. of patients	Before treatment		After treatment	
	range	mean	range	mean
Triglycerides (mMol/L)				
8	3.6-17.2	8.4	1.1-10.3	4.2
TG/HDL				
12	3.7-11.7	6.4	3.0-9.4	5.2
LDL/HDL				
8	0.8-4.3	2.2	0.65-3.1	1.5

Figure 4A

	Triglycerides		Cholesterol		LDL/Cholesterol		HDL/Cholesterol	
	Before	After	Before	After	Before	After	Before	After
1.	3.2	1,3	8.5	6.8	5.7	3.0	0.7	1.2
2.	1.3	1.3	7.1	7.7	5.3	5.7	1.2	1.4
3.	2.6	1.9	8.4	8.9	5.7	5.4	1.4	1.7
4.	2.4	2.2	5.9	5.1	3.5	3.2	1.1	1.3
5.	2.1	3.4	6.0	4.5	3.2	3.1	1.1	1.3
6.	1.1	1.3	6.5	6.5	3.7	3.8	1.1	1.2
7.	2.5	2.7	5.4	6.3	4.3	4.1	0.9	1.0
8.	2.1	2.0	7.6	7.2	4.9	4.6	1.7	1.8
9.	5.8	5.2	6.8	6.5	5.1	5.2	0.9	1.0
10.	1.2	1.2	5.2	5.4	3.8	3.5	0.9	1.2

Figure 4B

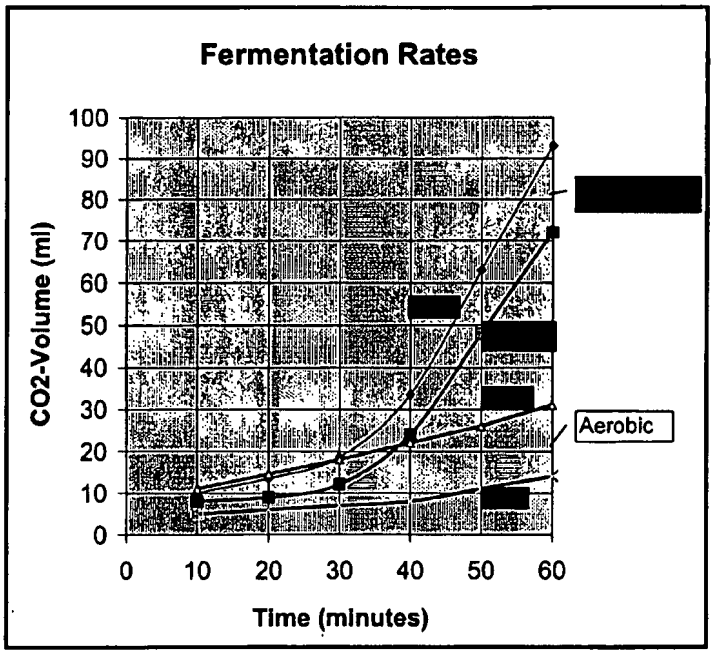
Fermentation Rates
CO2-Volume (ml)
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
Time (minutes)
Aerobic

Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/07527

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 47/00
US CL : 424/400, 424/439

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/400, 424/439

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,797,421 (ARIGA et al) 10 January 1989 (10.01.1989), coulmn 8, lines 43-68.	1-22
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A		23-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 May 2001 (22.05.2001)	26 JUL 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Charesse L. Evans Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/07527

Continuation of B. FIELDS SEARCHED Item 3: EAST, MEDLINE, BIOSIS, CAPLUS, USPATFULL, EMBASE, NAPALERT